Article

Methicillin-resistant *Staphylococcus aureus* (MRSA) contamination of retail pork

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Abstract — Recent reports of isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) from food animals have raised concern about the potential for foodborne transmission. This study evaluated the prevalence of MRSA contamination of retail pork from 4 Canadian provinces. Methicillin-resistant *Staphylococcus aureus* was isolated from 31/402 [7.7%, 95% confidence interval (CI): 5.5% to 10.7%] of samples. Adjusted for clustering at the provincial level, the prevalence was 5.8% (95% CI% 2.2% to 14.4%). The most common clone was Canadian epidemic MRSA (CMRSA)-5 (12/31, 39%), which has been widely identified in horses and horse personnel, but not in pigs. Ten of the 31 (32%) isolates were nontypable by pulsed-field gel electrophoresis (PFGE) and belonged to *spa* 539/t034, a clone that is associated with food animals internationally. Nine (29%) isolates were CMRSA-2, a common human epidemic clone that has been found in pigs in Canada. While the relevance of contamination of retail meat is currently unclear, further study is required to determine if food may be a source of infection.

Résumé — Contamination par *Staphylococcus aureus* résistant à la méthicilline (SARM) du porc de détail. Des rapports récents d'isolement de *Staphylococcus aureus* résistant à la méthicilline (MRSA) chez des animaux destinés à l'alimentation ont soulevé des préoccupations à propos du potentiel de transmission d'origine alimentaire. Cette étude a évalué la prévalence de contamination du porc de détail par le SARM dans 4 provinces canadiennes. Le *Staphylococcus aureus* résistant à la méthicilline a été isolé auprès de 31/402 [7,7 %, intervalle de confiance (IC) de 95 % : 5,5 % à 10,7 %] des échantillons. Rajustée pour le regroupement au niveau provincial, la prévalence était de 5,8 % (IC de 95 % : de 2,2 % à 14,4 %). Le clone le plus commun était le SARM épidémique canadien (SARMC)-5 (12/31, 39 %), qui a été communément identifié chez les chevaux et le personnel affecté au soin des chevaux, mais non chez les porcs. Dix des 31 (32 %) isolats étaient non typables par électrophorèse en champ pulsé (ECP) et appartenaient au spa 539/t034, un clone qui est associé aux animaux destinés à l'alimentation à l'échelle internationale. Neuf (29 %) isolats étaient SARMC-2, un clone épidémique humain commun qui se trouve chez les porcs au Canada. Bien que la pertinence de la contamination de la viande de détail soit actuellement imprécise, de nouvelles recherches sont requises pour déterminer si les aliments peuvent être une source d'infection.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant human pathogen, both for patients in hospitals and increasingly in otherwise healthy individuals in the community (1–3). Concurrent with the increase in community-associated (CA)-MRSA infections in humans has been the identification of MRSA in various animal species, including pigs (4–6). A link has been made between pigs and CA-MRSA infection and colonization in humans (6–9), almost exclusively involving 1 clone that can be found in a large percentage of pigs in some regions of Europe and North America (4–6,10). This clone is nontypable by *sma*I PFGE, it is sequence type (ST) 398 by multilocus sequence typing and consists of *spa* type 539/t034 or related types. Initial reports of a link between pigs and human MRSA have focused on direct contact with pigs or living in rural regions; however, an obvious concern about finding MRSA in food animals is the potential for contamination of meat. Concerns were first raised in a study from The Netherlands that reported isolation of MRSA from 2/64 (3.1%) raw retail pork samples and 0/15 beef samples, consisting of 1 ST398 strain and 1 USA300 (11). A subsequent Dutch study reported isolation of MRSA from 11.9% of various retail meat samples, including beef, pork, and chicken, with most isolates corresponding to ST398 (12). A smaller American study reported isolation of MRSA from 5.6% of pork and 3.3% of beef samples (13).

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Table 1. Summary of stratified random sampling selection method, with strata formed by the cumulative population quartiles from a list of divisions in the province sorted by population in ascending order

	Divisions selected	Sampling days/division/y
Ontario and Quebec		
Stratum 1	10	2
Stratum 2	4	5
Stratum 3	2	10
Stratum 4	1	20
British Columbia and Saskatchewan		
Stratum 1	9	2
Stratum 2	5	3
Stratum 3	2	5
Stratum 4	1	7

These studies raise concern about the potential for food to act as a vehicle for MRSA transmission. Broader studies using organized sampling schemes to avoid clustering and potential bias are needed in different regions to expand on these data. The objectives of this study were to determine the prevalence of MRSA contamination of retail pork in Canada and to characterize recovered MRSA isolates.

Materials and methods

Pork chops, ground pork, and pork shoulders were purchased at retail outlets in 4 Canadian provinces (British Columbia, Saskatchewan, Ontario, Quebec) between August and November 2008 inclusive, as part of the active retail surveillance component of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). Retail sampling is weighted by population. Using Statistics Canada data, 17 census divisions are randomly selected per province by stratified random selection. The strata are formed by the cumulative population quartiles from a list of divisions in a province sorted by population in ascending order, and stores within those divisions are sampled as described in Table 1. Field workers conduct 1 sampling day per week in Ontario and Quebec and biweekly sampling in the other 2 provinces. In each census division, 4 stores are selected prior to the sampling day based on store type. Three chain stores and 1 independent market or butcher shop are selected, except in densely populated regions where 2 chain stores and 2 independent markets or butcher shops are sampled to reflect the shopping behavior of the subpopulation. One sample of each commodity is obtained from each store. Where possible, stores are only sampled once per year.

Samples are purchased and shipped to the study laboratory on ice in their original packages via 24 h courier; they are processed within 72 h of receipt.

Pork chops and pork shoulders were tested by both direct and rinse methods. Direct culture involved removal of an approximately 2.5 cm^3 (15 g) section of meat using a sterile scalpel blade and inoculation into 50 mL of enrichment broth consisting of 10 g tryptone/L, 75 g sodium chloride (NaCl)/L, 10 g mannitol/L, and 2.5 g of yeast extract/L. After 24 h incubation at 37°C, 100 μL of broth was inoculated onto MRSA Chromogenic agar (BBL CHROMagar MRSA, Becton, Dickinson and Company, Sparks, Maryland, USA). The rinse method involved rinsing of the entire pork chop or pork shoulder in 50 mL of phosphate buffered saline (PBS, pH 7.4), inoculation of 1 mL of rinse solution into 9 mL of enrichment broth, and subsequent incubation as described. Ground pork was tested by inoculation of 15 g into 50 mL of enrichment broth, with subsequent handling as discussed. Isolates were identified as *S. aureus* by colony morphology, Gram stain appearance, catalase and coagulase reactions, and *S. aureus* latex agglutination test (Pastorex Staph-plus, Bio-Rad, Marnes-la-Coquette, France). Methicillin-resistance was confirmed by penicillin-binding protein 2a latex agglutination test (MRSA latex agglutination test, Oxoid, Hants, United Kingdom). A single MRSA colony from each positive sample was chosen for further study. Isolates were typed by PFGE (14). Isolates were also typed by sequencing of the X region of the protein A gene (*spa* typing) (15). For *spa* typing, sequences were analyzed using the eGenomics software (http://tools.egenomics.com). Ridom database equivalents were identified using the Ridom Spaserver Web site (www.spaserver.ridom.de). eGenomics *spa* types are reported using a numerical system (*spa* type 539) while Ridom *spa* types are reported using a numerical system preceded by a 't' (*spa* t034). Real-time polymerase chain reaction (PCR) was used to detect the *lukF* and *lukS* genes for Panton-Valentine leukocidin (PVL) (16).

Prevalence and 95% confidence intervals (95% CI) were calculated. Prevalence was also adjusted for clustering at the province level using generalized linear latent and mixed models (GLLAMM) with adaptive quadrature. The formula that was used to calculate the adjusted prevalence of MRSA was

$$P_{adj} = \frac{e^{\beta_0}}{1 + e^{\beta_0}} \quad \textit{Equation 1}$$

Where: β_0 is the coefficient for the intercept (17). A Chi-squared test was used for categorical comparisons, and $P < 0.05$ was considered significant for all analyses.

Results

Methicillin-resistant *S. aureus* was isolated from 31/402 (7.7%, 95% CI: 5.5% to 10.7%) samples. Adjusting for clustering at the provincial level, the prevalence was 5.8% (95% CI: 2.2% to 14.4%). There was a significant difference between provinces ($P < 0.001$), with MRSA isolated from 18/101 (18%) samples from British Columbia, 1/100 (1%) from Saskatchewan, 5/99 (5%) from Ontario, and 7/102 (7%) from Quebec. There was no significant difference between different products ($P = 0.99$) with MRSA isolated from 23/296 (7.8%) pork chops, 7/94 (7.4%) ground pork, and 1/12 (8.3%) pork shoulders.

Twenty-one (5.2%) samples were positive for MRSA using direct culture while 15/355 (4.2%) samples were positive using the rinse method. Nine samples were positive on direct culture but negative using the rinse method, while 10 others were positive only with the rinse method and only 5 were positive with both methods. Seven samples (ground pork) that were positive on direct culture were not tested using the rinse method.

Spa typing results are presented in Table 2. When related *spa* types were combined, there were 3 main clones, which corresponded to PFGE results. Twelve of 31 (39%) isolates consisting of 3 related *spa* types were classified as Canadian epidemic

Table 2. Typing results for methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from retail pork ($n = 31$)

PFGE clone (*n*, %)	*Spa* type	Motif	*n*	Source
CMRSA-5 (12, 8.4%)	7/t064	YHGCMBQBLO/r11r19r12r05r17r34r24r34r22r25	10 (32%)	BC (4), ON (3), QC (3)
	1/t008	YHGFMBQBLO/r11r19r12r21r17r34r24r34r22r25	1 (3.2%)	QC
	New	YHGCMBQBMO/r11r19r12r05r17r34r24r34r17r25	1 (3.2%)	BC
Nontypable (10, 32%)	539/t034	XKAOAOBQO/r08r16r02r25r02r25r34r24r25	10 (32%)	BC (7), SK (1), ON (1)
CMRSA 2 (9, 29.2%)	2/t002	TJMBMDMGMK/r26r23r17r34r17r20r17r12r17r16	4 (13%)	BC (3), QC (1)
	47/t045	TMDMGMK/r26r17r20r17r12r17r16	4 (13%)	BC (2), QC (2)
	New	TMEMGMK/r26r17r13r17r12r17r16	1 (3.2%)	ON (1)

BC — British Columbia; SK — Saskatchewan; ON — Ontario; QC — Quebec.

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MRSA-5 (CMRSA-5) by PFGE. This clone has previously been reported as being ST8 (18). Ten of 31 (32%) isolates were non-typable by PFGE and belonged to *spa* 539/t034, which is known to be ST398 (19). Nine (29%) isolates consisting of 3 related *spa* types were CMRSA-2, also known as USA100, which has been previously determined to be ST5 (18). No isolates possessed genes encoding for production of PVL. There were no apparent regional differences in *spa* type distribution.

Discussion

This study has determined that MRSA contamination of retail meat is not uncommon in Canada and is similar to that reported in other studies. While there was a significant difference in the prevalence between provinces, MRSA was found in pork products in every province. The reason for the difference in prevalence between provinces is unclear. Currently, the only study of MRSA in pigs in Canada involved farms in Ontario, and information about the prevalence of MRSA in pigs in other provinces is required to help interpret this finding.

The typing data were interesting and raise questions regarding the origin of contamination. The finding of PFGE nontypable *spa* 539/t034 MRSA, which has been previously identified as belonging to ST398, was expected as it is commonly reported in pigs internationally (4,6,10,20) and has been found in retail meat (11,12). It is reasonable to assume that ST398 contamination of meat is from pigs, either directly (deposition of MRSA from the animal onto meat during slaughter) or indirectly (environmental contamination resulting in subsequent food contamination). However, since ST398 MRSA colonization rates of pig farmers and pig veterinarians are high (6,10,21), it is also possible that slaughterhouse workers have high colonization rates, and that those individuals could have been the source of contamination, even though they may have ultimately acquired MRSA from pigs. Similarly, the finding of CMRSA-2, a common human epidemic clone in Canada (also known as USA100) was not surprising since this clone was commonly found in pigs in an Ontario on-farm study (10). It also accounted for 3/6 MRSA isolates in a recent American study of retail pork and beef (13). Pig, environmental, and human sources are potential origins of contamination by this strain and the fact that this is a common human strain will complicate assessment of the potential role of animals or food in human disease. Identification of CMRSA-5 was unexpected. This human epidemic clone has previously been reported as the predominant MRSA clone in horses and horse personnel in North America (22,23), but it has not been previously reported in pigs. Horses are not slaughtered at the same facilities as pigs in Canada, so cross-contamination at the slaughterhouse could not have occurred. It is possible that humans were the source of CMRSA-5 colonization; however, this is a relatively uncommon clone in the general population in Canada (18), apart from humans with horse contact. There is no information as to the prevalence of horse contact of slaughterhouse personnel and swine veterinarians in Canada tend to be swine specialists rather than mixed animal practitioners. While reports of MRSA in pigs in Europe tend to exclusively identify ST398, more diversity was present in the Ontario on-farm study. It is plausible that CMRSA-5 is actually present in the Canadian pig population, but was not detected in the earlier study because of its geographically limited nature. Further study of pigs in North America is required to help evaluate this finding, as is broader investigation of MRSA contamination of the environment at various levels of the food supply chain, and in humans working in those areas. This type of comprehensive "farm-to-fork" approach is required to assess the need for interventions and to develop measures to reduce contamination.

Another factor that requires consideration is the methodology that was used. Enrichment culture, as was employed here, is likely a very sensitive technique that could detect very low numbers of MRSA. While an infective dose is unknown, it is logical to assume that high levels of MRSA are more of a concern than very low numbers, and the methods used in this study do not differentiate those. The variability in results of rinse and direct culture methods on the same samples also requires further study, to determine whether this may reflect low or non-homogenous distribution of MRSA in meat or inherent differences in sensitivity of the different methods. Evaluation of methods for isolation and enumeration of MRSA in meat is required. The enrichment broth and selective agar that were used were chosen based on the authors' experience with them for various specimen types; however, there have been no objective evaluations of different media for isolation of MRSA from meat. Only a single MRSA colony was tested from each positive sample, so it is not known whether multiple strains could have been present in some samples. This would not have affected the overall prevalence but could have had an impact on the strain distribution. This is particularly true if one strain is able to grow better than others in the enrichment broth; however, information regarding inter-strain variation in growth in this broth is not known.

The public health relevance of MRSA in retail meat is entirely unclear. Staphylococcal food poisoning with MRSA has rarely been reported (24). Food poisoning caused by MRSA should be no different clinically than that caused by methicillin-susceptible

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S. aureus; however, it is possible that affected individuals could remain colonized if live MRSA was ingested along with staphylococcal enterotoxins. Another potential concern is meat as a vehicle for transmission of MRSA colonization. It is plausible that nasal MRSA colonization could occur if humans contaminate their hands by touching meat (or contaminated surfaces) then touching their nose before handwashing. This potential concern requires further study; however, standard recommendations for handling and cooking raw meat should greatly reduce if not eliminate the risk of transmission of MRSA, just as proper cooking and food handling should reduce or eliminate the risk of enterotoxin-associated gastroenteritis. It is also plausible that MRSA from meat could directly contaminate susceptible sites such as wounds, something that could be of particular concern to humans who work in the food preparation industry and may be prone to knife cuts on their hands. Further study of the potential role of meat in MRSA transmission in the community is required, including measures such as querying food contact history in studies of CA-MRSA.

While this study can certainly not implicate food as a source of human MRSA infection, the finding of MRSA in meat, including strains implicated in human infections, does raise concern and the possibility that food plays a role in the community spread of MRSA. Further study is required to better elucidate this possible role, and to determine if any mitigation strategies are appropriate.

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